ELECTROPHORESIS APPARATUS

BACKGROUND OF THE INVENTION

Preparative electrophoresis apparatuses normally comprise an upper electrode zone, a separation zone containing a cyclindrically shaped gel, an elution or collection zone and a lower electrode zone. The separation of the components, caused by the introduction of a potential gradient in the gel, results in the fact that the components are going to migrate with different speeds so that they leave the gel subsequently and enter into the elution zone. This zone is perfused by a buffered solution taking the components along to, for example, a fraction collector, from which they can be removed for further investigation.

A great variety in constructions of electrophoresis apparatuses is known, and much attention is paid to the shape of the elution chamber which has to fulfill several, partially conflicting, requirements. The absence of dead space and a relatively high flow speed are necessary to avoid the danger of contamination of fore-going fractions. The greater part of the apparatuses have their outlet at the side (see, e.g., P.H. Duesberg, Anal. Biochem. 11 (1965) 342). This is unfavourable because of the presence of a threshold at the outlet. The elution zone proposed by Schenkein (Anal. Biochem. 25 (1968), 387) has an outlet directed downward, thus avoiding the threshold.

A high flow speed of the buffered solution in the elution chamber necessary to obtain an as sharp separation as possible of the components, may result in an excessive dilution of the components, so that analysis thereof is made more difficult. This may be improved by decreasing the sizes of the elution chamber. Duesberg (loc. cit.) applied a conically shaped lower end of a mainly cylindrical gel, so that a smaller elution chamber was sufficient.

Further, the bottom of the separation zone generally consists of a semipermeable membrane, allowing the ions to migrate through it, thereby providing the electrical contact, but preventing the components of the material to be investigated (normally protein components) to pass.

However, membranes have disadvantages, for example, the so-called Bethe-Toropoff effect (H. Svensson, Adv. Protein Chem. 4 (1948) 251) and the possibility of denaturation or absorption. A sintered glass bottom is proposed in an apparatus described by Porath (Nature 182 (1958) 744) wherein a downward motion of the components of the sample is hindered by an upward flow of buffered solution.

It is an object of the invention to provide an electrophoresis apparatus wherein the above-mentioned advantages are combined with other advantages, as discussed hereinafter, and the disadvantages are eliminated.

SUMMARY OF THE INVENTION

The invention provides a preparative electrophoresis apparatus, comprising a funnel-shaped zone for the electrophoresis gel which is open at the upper side, a zone for circulating a first buffered solution thereabove containing a first electrode, said funnel-shaped zone at its lower end passing in a narrow aperture provided with a bottom enabling liquid to pass but to support the gel, said narrow opening being in connection with an elution chamber directly connected to an outlet, said elution chamber being further connected to a small conical jacket around the funnel-shaped zone, said conical jacket being connected with a zone containing a second electrode and being provided with an inlet for circulating a second buffered solution, said inlet being situated between the zone containing the second electrode and the elution chamber, said elution chamber further being provided with a downwardly directed outlet for the eluate, the apparatus further comprising means for cooling the liquids and the gel.

In this construction, there is no membrane for holding the components of the material to be investigated, at all, and this is not necessary since the second buffered solution is introduced between the elution chamber and the zone of the second electrode, so that by a proper adjustment of the flow speed of the second buffered solution all separated components are taken along without the danger of components migrating from the elution chamber to the second electrode and escaping from investigation. The absence of the membrane has a further advantage, i.e., absorption is avoided and electric effects of the membrane are absent. It is an advantage of the funnel-shaped separation zone that the sample may be applied in a relatively thin layer, causing a high separating power. Further, the elution chamber of the apparatus according to the invention is small, and the buffered solution is introduced in an annular manner, thus avoiding an inlet tube. This results in the advantage that there is no dead space, while the smallness of the elution chamber and the manner of introduction of the buffered solution further increase the separating power since mixing of fractions is avoided to a high extent, The volume of the elution chamber is small, it is about 0.01 to 1 ml, preferably 0.05 to 0.2 ml. The construction according to the invention allows a very efficient elution without the necessity of the use of an excessive amount of buffered solution, thus avoiding excessive dilutions which is of advantage for the further investigation of the eluate.

It is an additional advantage of the construction of the invention that the part of the gel where the electric field, and thus the heat formation, is greatest, i.e., at the lower end of the funnel-shaped zone, is most intensively cooled by the buffered solution since the flow speed of the buffered solution in that region of the conical jacket is highest.

It is an aspect of the invention to provide an apparatus wherein the apparatus consists of an upper part comprising a conically shaped part of the wall defining the funnel-shaped zone, an outer wall defining the circulating zone for the first buffered solution, and the first electrode, said upper part fitting to a lower part comprising the outer wall of the conical jacket, the second electrode, the inlet for the second buffered solution and at the lower end of the conical wall a somewhat diverging part cooperating with a plug provided with a central vertical passage, all in a manner such that the side walls of the elution chamber are formed by the lower part, the upper wall thereof by the upper part and the bottom is formed by the plug. This embodiment has the advantage that the sizes of the elution chamber are well defined and are still well approachable to be cleaned and to pour the gel in the upper part. It is appreciated that a carrier in powder form, such as Sephadex (trade mark) or glass powder, may be used. In an advantageous embodiment of the invention, the several parts are provided with ground glass joints (Schliff-